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Diagnostic performance of rapid diagnostic tests for the diagnosis of malaria at public health facilities in north-west Ethiopia

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Abstract

OBJECTIVE To assess the performance of RDTs against nested polymerase chain reaction (nPCR) for the diagnosis of malaria in public health facilities in north-western Ethiopia.

METHODS Cross-sectional study at public health facilities in North Gondar, Ethiopia, of 359 febrile patients with signs and symptoms consistent with malaria. Finger prick blood samples were collected for testing in a *P. falciparum*/pan-malaria RDTs and for molecular analysis. Sensitivity, specificity and predictive values were determined for the RDTs using nPCR as reference diagnostic method. Kappa value was determined to demonstrate the consistency of the results between the diagnostic tools.

RESULTS By RDTs, 22.28% (80/359) of patients tested positive for malaria, and by nPCR, 27.02% (97/359) did. In nPCR, 1.67% (6/359) and 0.28% (1/359) samples were positive for *P. ovale* and *P. malariae*, which had almost all tested negative in the RDTs. The sensitivity, specificity, positive and negative predictive values of RDTs for the diagnosis of malaria were 62.9%, 92.7%, 76.3% and 87.1%, respectively, with 0.589 measurement agreement between RDTs and nPCR. The sensitivity and specificity of RDTs for *P. falciparum* identification only were 70.8% and 95.2%, and 65.2% and 93.1% for *P. vivax*.

CONCLUSION Although RDTs are commonly used at health posts in resource-limited environments, their sensitivity and specificity for the detection and species identification of *Plasmodium* parasites were poor compared to nPCR, suggesting caution in interpreting RDTs results. Particularly, in the light of expanded efforts to eliminate malaria in the country, more sensitive diagnostic procedures will be needed.

keywords rapid diagnostic tests, nested polymerase chain reaction, diagnostic performance, Ethiopia

Background

Although malaria caused by *Plasmodium falciparum* remains the predominant species in Ethiopia, non-falciparum malaria is gaining importance. Close to one-third of malaria cases in Ethiopia are caused by *P. vivax*. *P. ovale* and *P. malariae* have only recently been identified as contributing a significant proportion of malaria cases [1, 2].

Malaria threatens the lives of 40% of the world's population [3]. Each year, there are estimated 350–500 million clinical cases, with about 90% of these occurring in sub-Saharan Africa [3–5]. Malaria is a leading communicable disease in Ethiopia: an estimated 57.3 million (68%) of the 84.3 million population live in areas at risk of malaria. The Federal Ministry of Health (FMOH) estimates that there are 5–10 million clinical malaria cases each year. In 2009/2010 health and health indicators report, 1581 malaria deaths were reported in Ethiopia [6].

In Ethiopia, the main species *P. falciparum* and *P. vivax* account for 60% and 40% of malaria cases, respectively [7], but only *P. falciparum* commonly

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causes severe disease in which the case fatality rate is about 10% in hospitalised adults and up to 33% in children under 12 years [8]. In addition to the health impact, malaria has a significant impediment to the socio-economic development of the country. Fertile lowlands and major river valleys have not been populated and developed largely due to the high malaria burden in these areas.

In Ethiopia, early diagnosis and prompt treatment are the main strategies in malaria prevention and control and to reduce morbidity and prevent mortality [9]. However, clinical diagnosis and empirical treatment have been the mainstay of malaria management in areas where laboratory facilities are not available. However, clinical diagnosis is notoriously unreliable and leads to overdiagnosis and overtreatment [10].

Although frequently not accessible in most peripheral health facilities in the country, Giemsa microscopy remains the gold standard for the laboratory diagnosis of malaria. However, it is time-consuming, requires trained personnel and needs careful preparation and application of reagents [11]. As a result, rapid diagnostic tests (RDTs) have been introduced for the diagnosis of malaria in remote areas where there are no microscopy facilities. However, poor and varying performances of RDTs have been reported in Ethiopia [12]. Therefore, this study aimed to assess the performance of RDTs as compared to nested polymerase chain reaction (nPCR), the most sensitive reference test, for the diagnosis of malaria at public health facilities in North Gondar, Amhara Regional State, Ethiopia.

Materials and methods

Setting and design

The study was conducted at Health Posts under Lamba Health Center, Maksegnet Health Center and Kola Diba Health Center in North Gondar, Amhara Regional State, Ethiopia. The altitude at these sites ranges from 1750 to 2100 m above sea level. According to the Municipal Health Bureau report, malaria is the most prevalent seasonal disease in these areas.

A health centre-based cross-sectional study was conducted from February to September 2014. Self-reporting febrile patients, in the age ranging from 1 year to 81 years, with signs and symptoms consistent with malaria attending either of the health posts were invited to participate. Patients who had received antimalarial drugs during the past 4 weeks prior to enrolment and patients unwilling to give consent and/or a blood sample were excluded from the study.

Study participants were recruited consecutively until a total of 359 patients with signs and symptoms consistent with malaria had been reached. Finger prick blood samples were collected from every participant and used to carry out RDT (CareStart™ Pf/Pan) analysis. CareStart™ Pf/Pan is targeted to detect *P. falciparum histidine-rich protein*-2 and plasmodial lactate dehydrogenase for the diagnosis of *P. falciparum* and other *Plasmodium* species, respectively.

Laboratory procedures

Two drops of blood from each participant were collected and transferred on filter paper (Whatman #903, GE Healthcare) labelled with the participant's study code and date. Each filter paper was dried individually to avoid any chance of contamination. The samples were then stored in small sealable plastic bags with desiccant and underwent molecular analysis at the laboratories of the Medical University of Vienna (MUV), Vienna, Austria.

A modified Chelex-based DNA extraction method using the InstaGene Whole Blood Kit (Bio-Rad Laboratories, Hercules, CA, USA) was used for the extraction and purification of *Plasmodium* DNA from the blood spots on filter paper. Parasite detection and species classification by nested PCR assay were performed for all samples as described previously [13, 14]. The individual interpreting the nPCR results was blinded to the results of RDTs.

Data analysis

Sensitivity, specificity and predictive values were determined using SISA online statistical software [15]. The kappa coefficient (Cohen's kappa coefficient as a measure of agreement for qualitative items) was determined to confirm the consistency of the results between the diagnostic tools.

Ethics

The study protocol was reviewed and approved by the Institutional Review Board of University of Gondar prior to the study. Informed consent and assent were obtained from all participants and/or their legal representatives after being translated and read in the vernacular language that the patients or the caretakers understood. Patients who tested positive for malaria by RDTs received immediate treatment according to the Ethiopian national treatment guidelines.

Results

Comparison of RDTs and nPCRs

Among the study participants, 22.28% (80/359) patients tested positive for malaria by RDTs of whom 12.53% (45/359), 5.85% (21/359) and 3.9% (12/359) were diagnosed as *P. falciparum*, *P. vivax* and mixed infection of *P. falciparum* and *P. vivax*, respectively. By nPCR, 27.02% (97/359) patients were malaria positive. Among RDT-negative samples, 12.9% (36/279) samples turned malaria positive by nPCR. In nPCR, the rate of mixed infection was 4.46% (16/359). The rates of *P. ovalae* and *P. malariae* infections were 1.67% (6/359) and 0.28% (1/359), respectively. Only one case of *P. ovalae* was reported as mixed infection of *P. falciparum* and *P. vivax,* while none of the *P. ovalae* and *P. malariae* infections were identified by RDTs (Table 1).

Diagnostic accuracy of RDTs compared to nested PCR for the diagnosis of malaria

Malaria rapid diagnostic tests had a sensitivity and specificity of 62.9% (95% CI: 50.6–75.1) and 92.7% (95% CI: 88.7–96.8), respectively, compared to the reference method, nPCR. Its corresponding positive and negative predictive values were 76.3% (95% CI: 64.4–88.1) and 87.1 (95% CI: 82.1–92.1). Overall, there was a moderate measurement agreement of test results between RDTs and nPCR (Kappa = 0.589).

RDTs had a sensitivity and specificity of 70.8% (95% CI: 56.7–84.9) and 95.2% (95% CI: 92.0–98.5) for the diagnosis and identification of *P. falciparum*, respectively. The corresponding sensitivity and specificity for the diagnosis and identification of *P. vivax* were 65.2% (95% CI: 40.4–90.0) and 93.1% (95% CI: 89.4–96.8), respectively.

Table 1 Comparison of RDTs and nested polymerase chain reaction for the diagnosis and species identification of malaria parasites, North Gondar, Amhara, Ethiopia, 2014

RDT (*n*)	nPCR (*n*)
P. falciparum (45)	*P. falciparum* (34); negative (11)
P. vivax (21)	*P. falciparum* (2); *P. vivax* (10); positive of genus *Plasmodium* (1); negative (8)
Mixed *P. falciparum* and *P. vivax* (14)	*P. falciparum* (8); *P. vivax* (1); mixed infection of *P. falciparum* and *vivax* (4); *P. ovalae* (1)
Negative (279)	*P. falciparum* (17); *P. vivax* (6); *P. ovalae* (5); mixed infection of *P. falciparum*, *vivax* and *malariae* (1); mixed infection of *P. falciparum* and *vivax* (1); positive for genus *Plasmodium* (6); negative (243)

n, number of blood samples tested; nPCR, nested polymerase chain reaction; RDTs, rapid diagnostic malaria tests.

Discussion

Early, prompt and particularly accurate malaria diagnosis down to species level even in remote areas is needed to provide prompt treatment and optimal case management of malaria [16, 17]. Standard diagnostic methods, such as Giemsa microscopy and RDTs, have become commonly available at most health facilities, even in resource-limited environments. However, both techniques have inherent limitations and show relatively poor performance with low parasite densities [11, 18–20]. The sensitivity of most RDTs used in routine diagnosis is strongly dependent on the parasite density and on the parasite species. The threshold of parasite density reliability giving positive results is also largely dependent on environmental factors, the brand of the RDTs, the targeted antigens, and a number of host factors. In previous publications the detection limits of RDTs has therefore been estimated at up to 200 parasites/μl of whole blood [21]. Non-falciparum malaria remains a major challenge for most RDTs with sensitivities reaching only non-satisfactory levels. In addition, many RDTs are not designed to adequately identify parasite species other than *P. falciparum*.

The combination of antibodies detecting *P. falciparum* antigens with antibodies detecting so called pan-malaria antigens is a common, economical, and to a certain extent highly valuable approach to designing RDTs. However, this does not allow for the identification of mixed infections and leaves non-falciparum malaria notoriously underdetected.

While non-falciparum malaria (mostly *P. ovale* and *P. malariae*) is not very common in most African countries, Ethiopia reports one of the highest *P. vivax* burdens in the world. *P. malariae* infections, however, seem to be uncommon in the region. In fact this is the first report of a confirmed *P. malariae* infection in this part of Ethiopia. There also seems to be a clear trend towards a relatively higher prevalence of non-falciparum due to a strong focus on falciparum malaria in the current malaria control programme, as seen previously, for example in South-East Asia [22–24]. Nevertheless, the elimination of *P. falciparum* is a goal in itself, and would be a major achievement even if that of the other species would take much longer. In this setting, the accurate diagnosis of non-falciparum malaria, preferably down to species level is therefore of utmost importance. This particularly applies to

elimination settings in which non-falciparum malaria is likely to play an important role due to the inherent challenges associated with eliminating relapsing malaria.

The present study adds relevant information regarding the performance of currently used malaria RDTs in the study area. RDTs showed a sensitivity of only 62.9% and specificity of 92.7% regardless of *Plasmodium* species when using nPCR as a reference method. In practice, this means that more than 10% of all patient samples would have been missed as being malaria positive. The utilisation of *Plasmodium* genus and species-specific markers makes nested PCR an ideal confirmatory test for malaria diagnosis as it also allows for the detection of low density infections and even more importantly of mixed infections, which could not be identified by the RDTs used in this study [13].

A similarly poor performance of malaria RDTs has previously been reported from Gabon, Nigeria and China–Myanmar [25–27]. A study from Zambia reported that community health workers read faint positive and invalid results of RDTs as negative suggesting that subjective interpretation may contribute to poor performance [28]. An earlier study from Angola suggested that performance may also be related to the age of the patients with malaria [29]. In the current study, 57.4% of the study participants were 18 years and older. Accordingly, the sensitivity of RDTs in study participants under 18 years (72.9%) was higher than older ages (50.0%). The fact that previous studies in Ethiopia and Burkina Faso reported a relatively good performance of malaria RDTs compared to Giemsa microscopy [30, 31] may have been influenced by the inherent limitations of Giemsa microscopy rather than good performance of RDTs [22].

In the current study, the RDTs consistently showed better performance in *P. falciparum* than in *P. vivax* (sensitivity 70.8% *vs.* 65.2% and specificity 95.2% *vs.* 93.1%). Similar results have been reported previously, for example from Belgium and the China–Myanmar border area. Throughout most studies the key factors contributing to the performance of RDTs are parasite density and *Plasmodium* species.

A factor strongly influencing the performance of HRP2-based RDTs in certain regions of the world seems to be the genetic variability of the *PfHRP2* gene which results in *P. falciparum* infections not being detected in spite of relatively high parasite densities [32]. This has previously been reported from a number of countries in Latin America [33, 34]. In how far this could influence RDTs results in Ethiopia remains to be seen.

The relatively poor performance of RDTs in our study underlines the urgent need for new diagnostic tests that are highly sensitive and specific but also economical and field deployable. Nested PCR, which was used as the reference test in this study, may provide adequate performance but is clearly not suited for use in resource-limited environments. Loop-mediated isothermal amplification (LAMP), a relatively recent development that considerably simplifies molecular analysis, may be a major step towards making molecular techniques available in such settings. In the meantime, the performance of RDTs could profit from improved training for healthcare workers and from optimising integrated diagnostic approaches with the ultimate goal of supporting malaria elimination programmes in Ethiopia [35–37].

One limitation of this study is that malaria microscopy was not performed and parasite density therefore not reported.

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